09/926036

FIGURE 1

```
GAATTCCGGGCCTTTTATTGTTCCTAGCCCTTTAAGGGCAAGGGGCTCTGCTGGGCTTGGAAAAATGCCTTCTTCAATT    80
                                                                  M  S  F  F  N
TCCGTAAGATCTTCAAGTTGGGGAGCGAGAAGAAGAAGAAACAGTACGAACACGTGAAGAGAGACCTGAACCCCGAAGAG   160
 F  R  K  I  F  K  L  G  S  E  K  K  K  K  Q  Y  E  H  V  K  R  D  L  N  P  E  E
TTTTGGGAGATTATTGGAGAACTGGGCGACGGAGCCTTCGGGAAAGTCTATAAGGCCCAGAATAAAGAGACCAATGTTTT   240
  F  W  E  I  I  G  E  L  G  D  G  A  F  G  K  V  Y  K  A  Q  N  K  E  T  N  V  L
AGCTGCTGCAAAGGTGATTGACACCAAATCTGAAGAAGAGCTTGAAGATTATATGGTTGAGATTGACATATTAGCATCTT   320
  A  A  A  K  V  I  D  T  K  S  E  E  E  L  E  D  Y  M  V  E  I  D  I  L  A  S
GTGATCACCCAAACATCGTTAAGCTTCTAGATGCCTTCTATTACGAGAACAACCTTTGGATCCTCATTGAATTCTGTGCA   400
C  D  H  P  N  I  V  K  L  L  D  A  F  Y  Y  E  N  N  L  W  I  L  I  E  F  C  A
GGGGGAGCAGTGGATGCTGTGATGCTTGAACTTGAGAGACCATTAACTGAATCCCAAATCCAAGTAGTCTGCAAGCAGAC   480
 G  G  A  V  D  A  V  M  L  E  L  E  R  P  L  T  E  S  Q  I  Q  V  V  C  K  Q  T
ATTAGAGGCATTGAATTACTTACATGACAATAAAATCATCCACCGAGATCTAAAAGCTGGCAATATTCTCTTTACCTTAG   560
  L  E  A  L  N  Y  L  H  D  N  K  I  I  H  R  D  L  K  A  G  N  I  L  F  T  L
ATGGAGACATTAAATTAGCGGATTTTGGAGTATCAGCTAAAAATACCAGGACAATTCAAAGGAGGGATTCATTTATTGGC   640
D  G  D  I  K  L  A  D  F  G  V  S  A  K  N  T  R  T  I  Q  R  R  D  S  F  I  G
ACACCATATTGGATGGCCCCTGAAGTAGTCATGTGTGAGACATCAAAAGACAGACCTTATGACTACAAAGCTGATGTTTG   720
 T  P  Y  W  M  A  P  E  V  V  M  C  E  T  S  K  D  R  P  Y  D  Y  K  A  D  V  W
GTCCCTGGGTATTACTTTAATTGAAATGGCTGAGATAGAGCCACCTCATCATGAGTTAAATCCAATGCGGGTTCTGCTGA   800
  S  L  G  I  T  L  I  E  M  A  E  I  E  P  P  H  H  E  L  N  P  M  R  V  L  L
AAATTGCAAAATCTGAGCCCCCAACATTAGCACAGCCATCAAAATGGTCTTCAAATTTTAAGGACTTTCTAAGGAAATGC   880
K  I  A  K  S  E  P  P  T  L  A  Q  P  S  K  W  S  S  N  F  K  D  F  L  R  K  C
TTGGAAAAGAATGTGGATGCCCGGTGGACCACGTCTCAGCTGTTGCAGCATCCCTTTGTTACCGTTGATTCCAACAAACC   960
 L  E  K  N  V  D  A  R  W  T  T  S  Q  L  L  Q  H  P  F  V  T  V  D  S  N  K  P
AGTCCGAGAGTTGATTGCTGAGGCAAAGGCTGAAGTAACAGAAGAAGTTGAAGATGGCAAGGAAGAAGATGAGGAGGAGG  1040
  V  R  E  L  I  A  E  A  K  A  E  V  T  E  E  V  E  D  G  K  E  E  D  E  E  E
AAGCAGAGAATGCTCTGCCAATACCTGCAAATAAACGTGCCTCCTCTGACCTCAGCATTGCCAGCTCTGAAGAAGATAAA  1120
E  A  E  N  A  L  P  I  P  A  N  K  R  A  S  S  D  L  S  I  A  S  S  E  E  D  K
CTTTCACAAAATGCTTGTATTTTGGAATCTGTGTCAGAAAGAACAGAACAAAGTACTTCTGAGGAAAAATTTAGCAACAA  1200
 L  S  Q  N  A  C  I  L  E  S  V  S  E  R  T  E  Q  S  T  S  E  D  K  F  S  N  K
AATTCTTAATGAGAAACCTACGACTGACGGTCCTGAGAAGGCTGTGGATGAGCATGCAAGTGATGTGAACTTAGAAACTG  1280
  I  L  N  E  K  P  T  T  D  G  P  E  K  A  V  D  E  H  A  S  D  V  N  L  E  T
GGGCTGAACTAAATGACCAAACAGTAGGAATCCATGAGAATGGGAGAGAGAAGAAAAGACCCAAGCTGGAAAATCTGCCA  1360
G  A  E  L  N  D  Q  T  V  G  I  H  E  N  G  R  E  K  K  R  P  K  L  E  N  L  P
GATACACAAGACCAGCAAACTGTGGATGTTAATTCAGTCAGTGAAGAAAATGAGAATAATAGAGTAACTTTAGAAACGAA  1440
 D  T  Q  D  Q  Q  T  V  D  V  N  S  V  S  E  E  N  E  N  N  R  V  T  L  E  T  N
CACTGATTGTCTGAAACCAGAGGAAGACAGAAATAAAGAAAACCAAGAGACACTTGAGAGTAAACTTATACAATCTGAAG  1520
  T  D  C  L  K  P  E  E  D  R  N  K  E  N  Q  E  T  L  E  S  K  L  I  Q  S  E
AAATTAATGACACACATACAAACAATGGACTTAGTTTCTCAAGAGACTGGAGAAAAAGAAGCAGATTTTCAGGCAGTT  1600
E  I  N  D  T  H  I  Q  T  M  D  L  V  S  Q  E  T  G  E  K  E  A  D  F  Q  A  V
GACAATGAAGTTGGGCTTACAAAGGAAGAAACCCAAGAGAAATTAGGAAAAGATGGTACAGCTCAAAAAGTTATAACCAG  1680
 D  N  E  V  G  L  T  K  E  E  T  Q  E  K  L  G  K  D  G  T  A  Q  K  V  I  T  S
TGATAGAAGCAGTGAGGTGGGGACAGACGAGGCTCTAGATGACACTCAGAAGGCTGCTGAGCTCAGTAAGGCAGCACAGA  1760
  D  R  S  S  E  V  G  T  D  E  A  L  D  D  T  Q  K  A  A  E  L  S  K  A  A  Q
GTGGGGAAGGGGACGAAGCCCTGGCGCCTACCCAGACACTAGCAGAGAAGCCCACAGAGGGCCCTGAGGCCGGTGGGGCT  1840
S  G  E  G  D  E  A  L  A  P  T  Q  T  L  A  E  K  P  T  E  G  P  E  A  G  G  A
GAGGAAGAGCCTCCTGGTGGAGAGAGAGTTGAGGATAAACAGCCAGAGCAGCAGCCTGCAGTGTGTGAAGCTGAGGGACA  1920
 E  E  E  P  P  G  G  E  R  V  E  D  K  Q  P  E  Q  Q  P  A  V  C  E  A  E  G  Q
GTTAACCAGCACGTCAGAGACCACACGGGCAACCCTGGAGCAACCGGAGACGGATGAAGTTGAGCAGGTCAGCGAGTCCA  2000
  L  T  S  T  S  E  T  T  R  A  T  L  E  Q  P  E  T  D  E  V  E  Q  V  S  E  S
ATAGCATTGAGGAGCTAGAGAGACTTGTAGTTACTGGTGCTGAGGCACGGGCTCTCGGGAGTGAAGGTGAGGCAGCTGCT  2080
N  S  I  E  E  L  E  R  L  V  V  T  G  A  E  A  R  A  L  G  S  E  G  E  A  A  A
ACTGAGGTAGATTTGGAGAGAAAAGAAAACGCACAGAAAGTGCCCGTTAAAGCAGAGTCCCAAGCTCCTGCAGCATCGCA  2160
 T  E  V  D  L  E  R  K  E  N  A  Q  K  V  P  V  K  A  E  S  Q  A  P  A  A  S  Q
GCCCAGCGAGCCTCACCCTGTCTTAATACCCAGTATTAATATTAATTCTGAAACCACAGAAAATAAAGAAGAAATGGGTG  2240
  P  S  E  P  H  P  V  L  I  P  S  I  N  I  N  S  E  T  T  E  N  K  E  E  M  G
CTTTGCCAAAACCTGAAACCATACTGCCACCAGAGCCTGAACATGAAAAGGGAAATGACACCGACTCAGGGACTGGGTCC  2320
A  L  P  K  P  E  T  I  L  P  P  E  P  E  H  E  K  G  N  D  T  D  S  G  T  G  S
ACTGTGGAGAATAGCAGTGGTGACCTGAACTTGTCCATCTCTAGCTTCCTAAGCAAAGCTAAGGACAGCGGCTCAGTGTC  2400
 T  V  E  N  S  S  G  D  L  N  L  S  I  S  S  F  L  S  K  A  K  D  S  G  S  V  S
TCTGCAGGAGACAAGAAGACAGAAGAAAACATTGAAGAAAACCAGAAAGCCAACTGTCGATGGTGTAGAAGTGAGTGTGA  2480
  L  Q  E  T  R  R  Q  K  K  T  L  K  K  T  R  K  P  T  V  D  G  V  E  V  S  V
CGACATCGAAGATAGTTACAGACAGCGACTCCAAAACGGAGGAACTGCGCTTTCTCAGGCGTCAGGAACTTCGGGAGGTG  2560
T  T  S  K  I  V  T  D  S  D  S  K  T  E  E  L  R  F  L  R  R  Q  E  L  R  E  V
AGGCTTCTTCAGAAGGAGGAGCAGCGAGCCCAGCAGCAGCTCAATGGGAAACTGCAGCAGCAGCGGGAGCAGATCTTCAG  2640
R  L  L  Q  K  E  E  Q  R  A  Q  Q  Q  L  N  G  K  L  Q  Q  Q  R  E  Q  I  F  R
GCGCTTTGAGCAGGAGAAGCTGAGCAAGAAGAGGCAGTATGACCAAGAAATTGAGAAGATGAAGAAACAGCAGAAACAGC  2720
  R  F  E  Q  E  K  L  S  K  K  R  Q  Y  D  Q  E  I  E  K  M  K  K  Q  Q  K  Q
AATTGAAGCGGCTAGAACAACAGTACACTAAGCGGCTGAGAGAGGCAGCCAAGGCGATCAAAGGAGAGCAGGAGAAGGAG  2800
Q  L  K  R  L  E  Q  Q  Y  T  K  R  L  R  E  A  A  K  A  I  K  G  E  Q  E  K  E
CTGTCCAAGTTCCAGAATGTGCTGAAAAACCGCAAGAAGGAGGAACAAGAATTTGTTCAGAAGCAACAACAAGAGTTAGA  2880
 L  S  K  F  Q  N  V  L  K  N  R  K  K  E  E  Q  E  F  V  Q  K  Q  Q  Q  E  L  D
TGGTTCTCTGAAAAAGATTATCCAGCAGCAGAAGGCAGAGTTGGCCAATATTGAGAGAGAATGCCTGAATAACAAGCAGC  2960
  G  S  L  K  K  I  I  Q  Q  Q  K  A  E  L  A  N  I  E  R  E  C  L  N  N  K  Q
AGCTCATGAGAGCTCGAGAAGCCGCAATTTGGGAGCTTGAAGAGCGACATTTACAAGAGAAGCACCAGCTGCTTAAACAG  3040
Q  L  M  R  A  R  E  A  A  I  W  E  L  E  E  R  H  L  Q  E  K  H  Q  L  L  K  Q
CAGCTTAAAGATCAGTATTTCATGCAGAGACATCAGCTGCTAAAACGCCATGAGAAGGAAACAGAACAAATGCAGCGCTA  3120
 Q  L  K  D  Q  Y  F  M  Q  R  H  Q  L  L  K  R  H  E  K  E  T  E  Q  M  Q  R  Y
CAATCAACGACTTATTGAAGAACTGAAAAACAGACAGACTCAGGAACGAGCGAGACTGCCCAAGATTCAAAGAAGTGAAG  3200
  N  Q  R  L  I  E  E  L  K  N  R  Q  T  Q  E  R  A  R  L  P  K  I  Q  R  S  E
CCAAGACACGAATGGCCATGTTTAAAAAAAGTTTGAGGATCAACTCAACAGCCACACCAGACCAGGACCGTGAAAAAATT  3280
A  K  T  R  M  A  M  F  K  K  S  L  R  I  N  S  T  A  T  P  D  Q  D  R  E  K  I
AAACAGTTTGCTGCACAAGAAGAAAAGAGACAGAAAAATGAGAGAATGGCTCAGCATCAAAAACATGAGAGCCAAATGCG  3360
 K  Q  F  A  A  Q  E  E  K  R  Q  K  N  E  R  M  A  Q  H  Q  K  H  E  S  Q  M  R
GGATCTTCAGTTGCAGTGTGAAGCCAATGTTCGGGAACTGCACCAGCTGCAGAATGAAAAATGCCACCTGTTGGTTGAAC  3440
  D  L  Q  L  Q  C  E  A  N  V  R  E  L  H  Q  L  Q  N  E  K  C  H  L  L  V  E
ATGAGACTCAGAAGCTGAAGGAGTTGGATGAGGAGCACAGCCAAGAGCTGAAGGAGTGGAGAGAGAAGCTGAGACCCAGG  3520
H  E  T  Q  K  L  K  E  L  D  E  E  H  S  Q  E  L  K  E  W  R  E  K  L  R  P  R
AAGAAGACACTGGAAGAAGAGTTTGCCAGGAAACTGCAGGAACAGGAAGTGTTCTTTAAAATGACTGGGGAGTCCGAATG  3600
 K  K  T  L  E  E  E  F  A  R  K  L  Q  E  Q  E  V  F  F  K  M  T  G  E  S  E  C
TCTTAATCCATCAGCACAGAGCCGGATCTCTAAATTCTACCCTATTCCCACCTTACATTCCACTGGGTCATTAGAACAGC  3680
  L  N  P  S  A  Q  S  R  I  S  K  F  Y  P  I  P  T  L  H  S  T  G  S  .
AAGTGTCCTCATCTGGATTTGGCTTCTAAGTACATCATTGTATTCTCTTCATCTTCCACAGTATGTATGACTACAAAGAC  3760
AATCACCTGCTTCATCTTCTTGGTGGTTTTAAAAATTTCTTTCTTGAATTTTATTGTTAAACAAAGATGAAGGGCAGACG  3840
AACTAAGACAGATGCTCGGCCATGTTGGTGACGTAGCATCTCGTGGTAATTCCCTAAGGTGATTTTGTATATTGACCTTA  3920
AATATTGTATTCTTTAGACACTGTTATTGAAAACTGCCAGAGACATAATGTTTAAAGTTATTTGGAAAATATATCTGTTA  4000
CATCACTAAGTATTAATAAATATTGTTTTACCTGATTTCTCAATGATGCTAAATTCTATAGAAAGGACTCTGCTATAGAA  4080
TTGGGATAATTTTCTTTTGGTAAACCAACTCTTACTTTTAAAAAGCCATGAGTTAGAGAATACGTTATTGATTCAGTACA  4160
TAGATATATTTTTATCACTAAACAGGATCAAAATCTTTTAAAAGAGAAAAGTTATTTCAATAATTTGTCGCAAACATTAG  4240
CCTGTGTGGGTAGGAGCTAATTATTAAGGTGCTAATTTTATTAAGATAGTGCCTAAAACACTAAATTTTAAACATATGTA  4320
AAATGGGGCTTTCCTTTTGGTCATGAGGAACAAAGTCCCCTTCTCACTGACATCACTGTTTTAAAAACTTGCTTATATTA  4400
CCATTCCGTAGAAAATTACATCCTAAAACAGATGGTGTACAAAGCCTGGGAAGATGGGAGAACTGGCTTTAACATGTGAG  4480
TTGGTGAGCCCCTTTACTCTCTGAGAGATGCCCACAGCTCAGGGAGTTCTGCCATCACCGAGAGCTCAGCCCTGAGCTGC  4560
AGTCACGGCTACAGTTCTGAAGCTAGTCTCAGCAGTGTTTGTCCACAATGGATTTTCTTTCATATCAGACACACACCCAA  4640
GGTGACACTGGGTTGTTTCCAATGAGGTTATTCAAGTGCATTTTGGTTTTATTTTATTCCATGTTTAAATTAATTACTTT  4720
GGGTAGGAAAAGCCTGAAGTCTCTATCATGTGGTTACTGGTACATGTGATTTTGATGAAATGTTCACTCTTAGTTCCTTT  4800
TGAAGTTCAGAATCTCAGCCACATGTCAGGTCAACAGTGTTAGAAACAGCACATTTGTAAATGATGCTCACTGCCTGCCA  4880
TTGGCAACCTGTTCTCTTCTAAGAGTGCTGGGTACCAAATTGTTACCAGTATTTAGTTATATTTTGAGGTTGGTGAAAAG  4960
TAGTGTAATGTGGGAGCAGGAGTTCATGTAAGAACCGTAGATGTCTGTAGAGCTTTCTGGCCCGTTTGTTTGAAACTTTGG  5040
ATGCTGAGCTCTATCTGGTTAGATGGTTTAAAAATGCATGTGTAATTTTAATTTTATAATTATTTTGGCAAGCATAATTT  5120
TTTCTGGACAACCTTGTAGGTAGCCTTAACTTTTAGCCAACTTTGTTTTTTATATAAATATATATAAATATATACATATA  5200
TAATGTATGGTTGTAAATTCATACACTTATCACATGATGTGTTACTATATACA GAATTC                       5253
```

```
                                          M S F F N     5
F R K I F K L G S E K K K K Q Y E H V K R D L N P E E   32
  P W E I I G E L G D G A F G K V V Y K A Q N K E T N V L   59
A A A K V I D T K S E E E E L E D Y M V E T D I L A S   85
C D H P N I V K L L D A F Y Y E N N L W I L I E F C A   112
G G A V D A V M L E L E R P L T E S Q I Q V V C K Q T   139
L E A L N Y L H D N K I I H R D L K A G N I L F T L   165
D G D I K L A D F G V S A K N T R T I Q R R D S F I G   192
T P Y W M A P E V V M C E T S K D R P Y D Y K A D V W   219
S L G I T L I E M A E I E P P H H E L N P M R V L L   245
K I A K S E P P T L A Q P S E W S S N F K D F L K K C   272
L E K N V D A R W T T S Q L L Q H P F V T V D S N K P   299
V R E L I A E A K A E V T E E V E D G K R E D E E E   325
E A E N A L P I P A N K R                               352
                                                        379
                                                        405
                                                        432
                                                        459
                                                        485
                                                        512
                                                        539
                                                        565
                                                        592
                                                        619
                                                        645
                                                        672
                                                        699
                                                        725
                                                        752
                                                        779
                                                        805
                                                        832
                                                        859
                                                        885
                                                        912
                          E Q E F V Q K Q Q Q E L D     939
  G S L K K I I Q Q Q K A E L A N I E R E C L N N K Q   965
Q L M R A R E A A I W E L E E R R H L Q E K H Q L L K Q  992
Q L K D Q Y F M Q R H Q L L K R H E K E T E Q M Q R Y  1019
N Q R L I E E L K N R Q T Q E R A R L P K I Q R S E   1045
A K T R N A M F K K S L R I N S T A T P D Q D R E K I  1072
K Q F A A Q E E K R Q K N E R M A Q H Q K H E S Q M R  1099
  D L Q L Q C E A N V R E L H Q L Q N E K C H L L V E  1125
H E T Q K L K E L D E E H S Q E L K E W R E K L R P R  1152
K K T L E E E P A R K L Q E Q Z V F F K M T G E S E C  1179
  L N P S A Q S R I S K F Y P I P T L H S T G S .      1202
```

FIGURE 3A

FIGURE 3B

FIGURE 3C

FIGURE 4A

FIGURE 4B

FIGURE 4C

FIGURE 4D

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FIGURES 5A-B

A

C2C12
days in DM   293/SMAK 293/SMAKΔ3'

M   0   1   3   5

221 -

133 -

93 -

67 -

56 -

B

                    C2C12 P19   10%FCS 0.5%FCS/24h +10%FCS/24h

M   NIH3T3

221 -

133 -

93 -

67 -

56 -

FIGURES 6A-B

FIGURES 7A-C

A

1 2 3 4 5 6 7 8 9 10

◄ SMAK

◄ H1

IP Kinase Assay

B

◄ p220/SMAK

Immunoblot

C

IP JNK1

293 +UV +SMAK

GST-Jun ►

IP ERK1/2

293 +EGF +SMAK

MBP ►
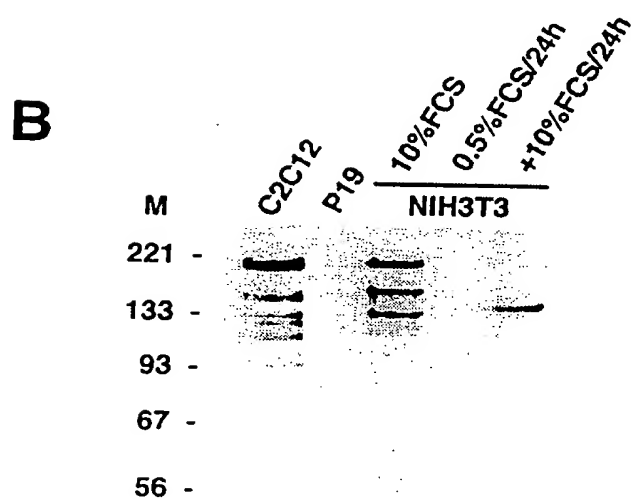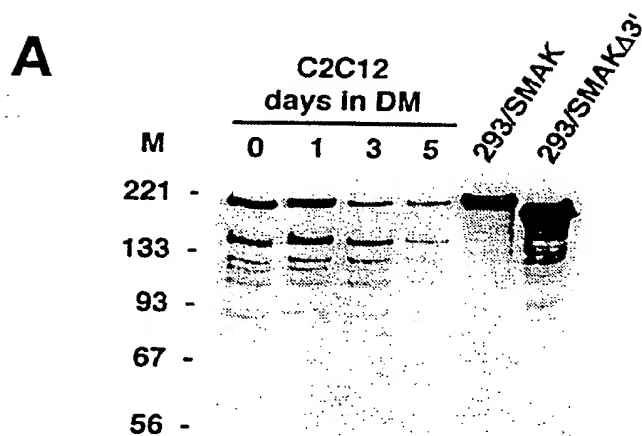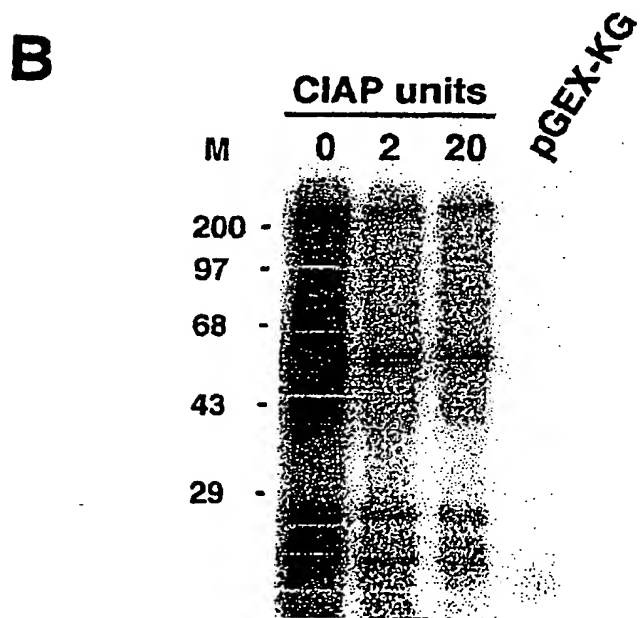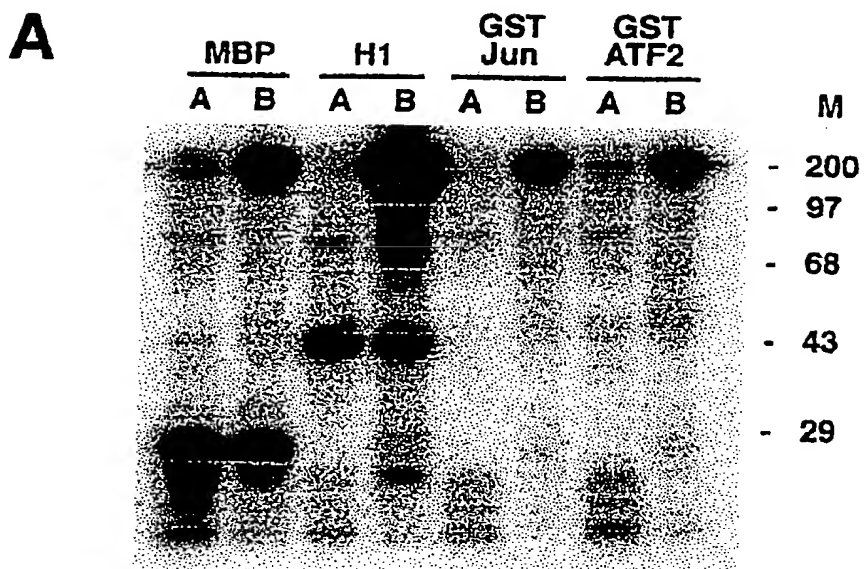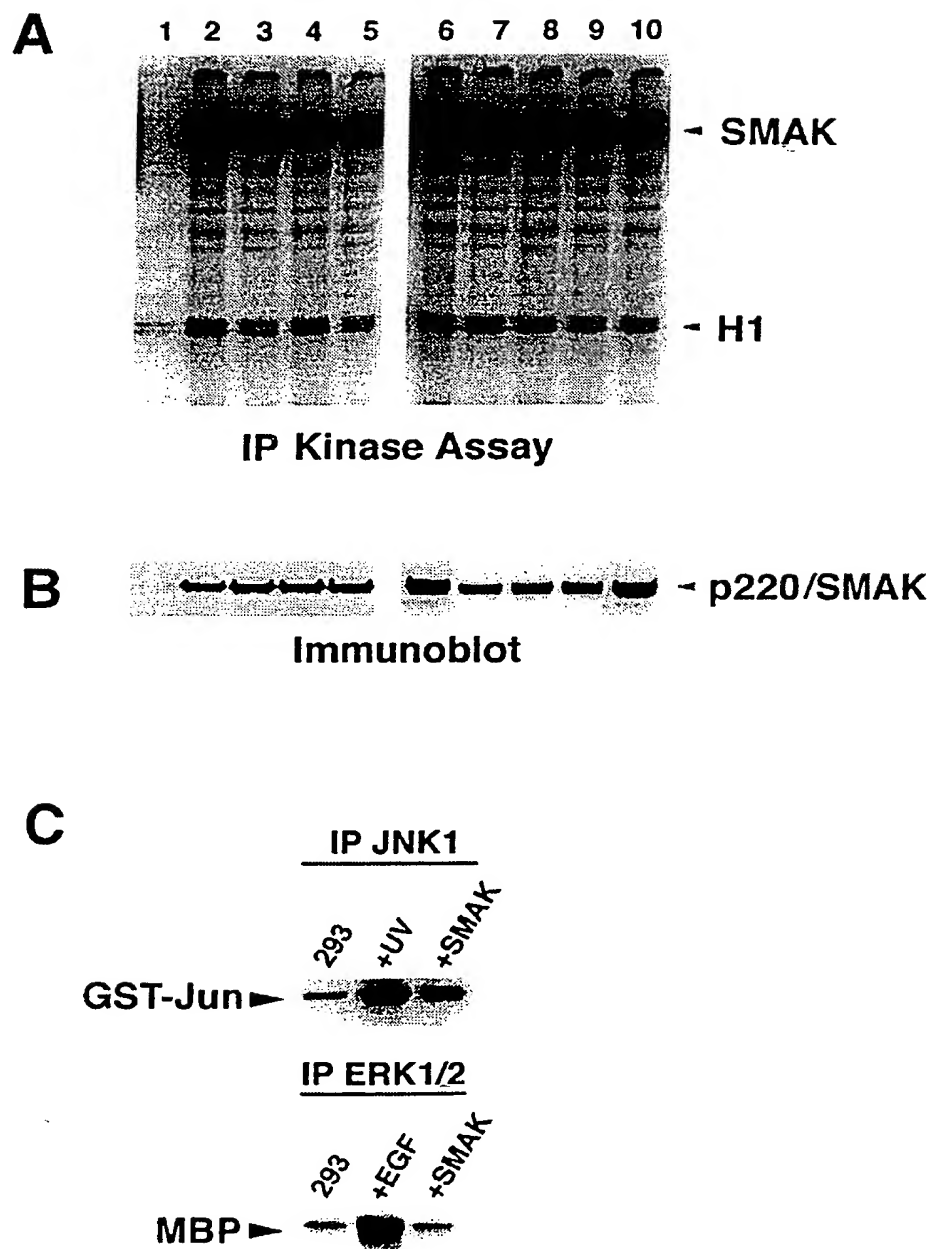
FIGURES 8A-B

Anti-Myc

SMAK

SMAK^{K63R}

FIGURES 9A-H

SMAK   Phase

SMAK   Annexin V

K63R   Annexin V

SMAK   TUNEL
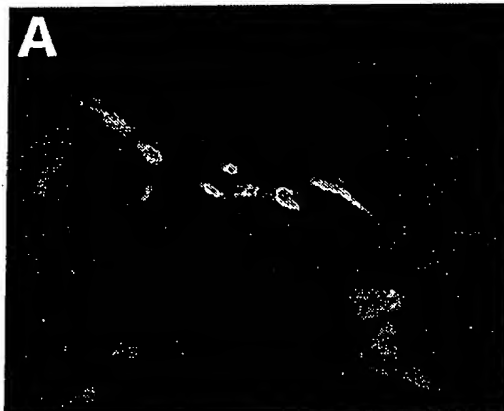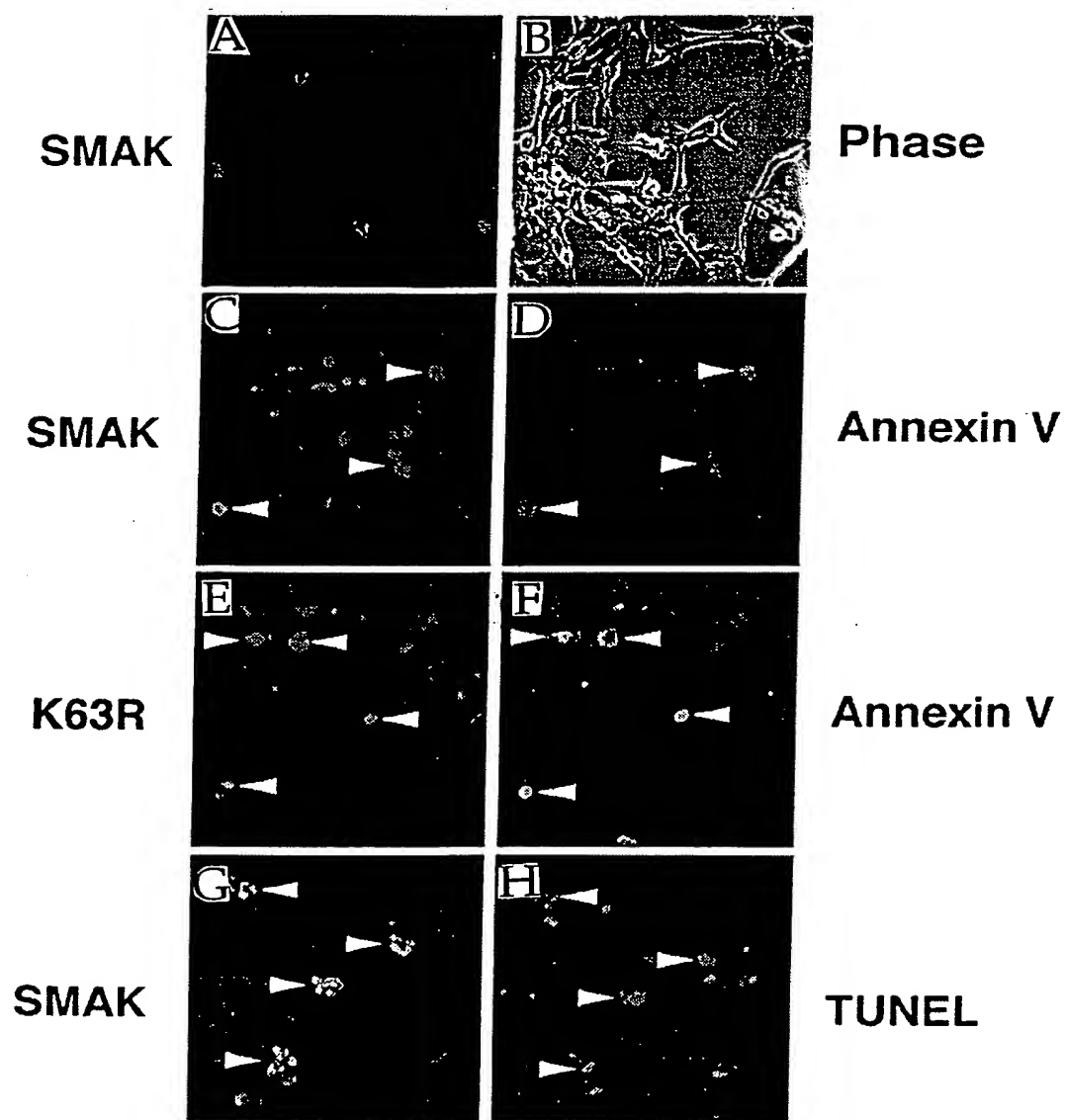
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FIGURE 10

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FIGURES 11A-F

FIGURES 12A-L

SMAK (A)

p65 PAK (B)

RhoA^{N19} (C)

Rac1^{V12} (D)

RhoA^{V14} (9E10) (E)

RhoA^{V14} (Phase) (F)

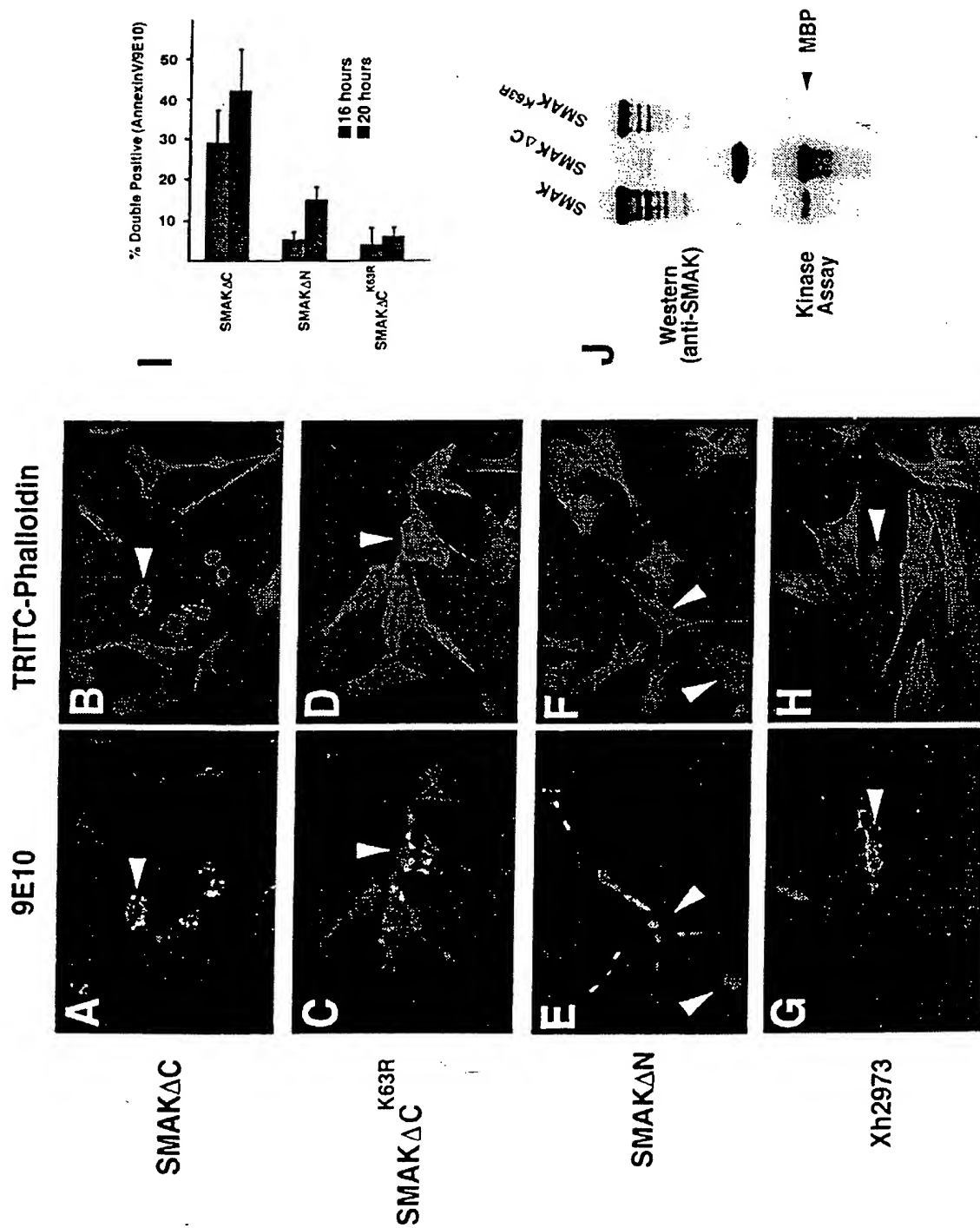HA-SMAK (G)   Myc-RhoA^{N19} (H)   HA+Myc (I)

HA-SMAK (J)   Myc-PAK (K)   HA+Myc (L)
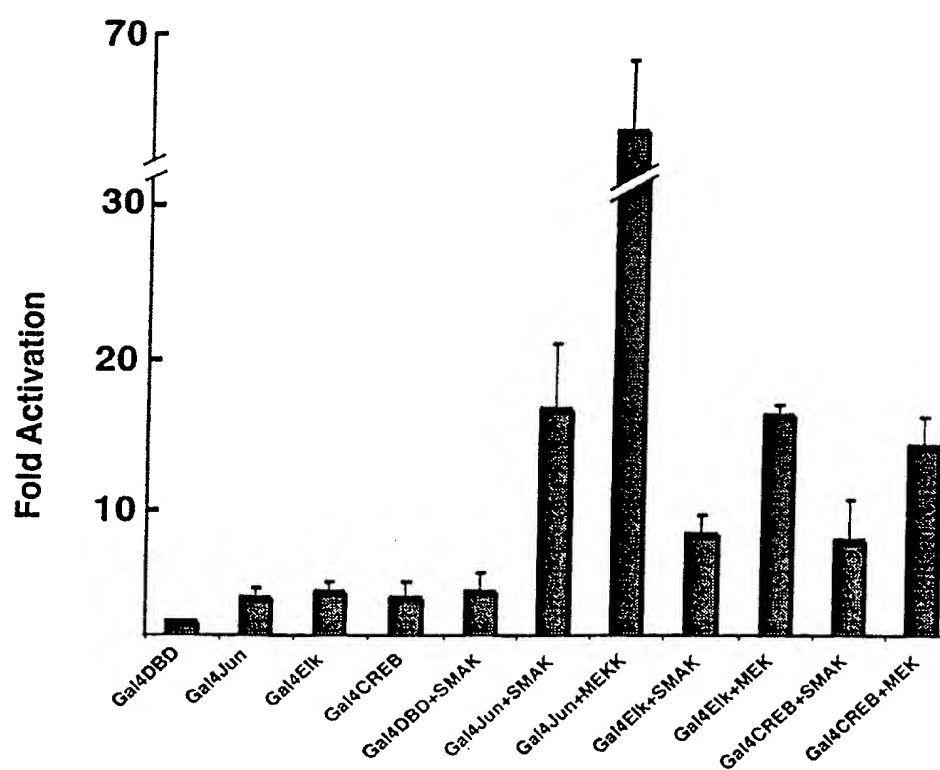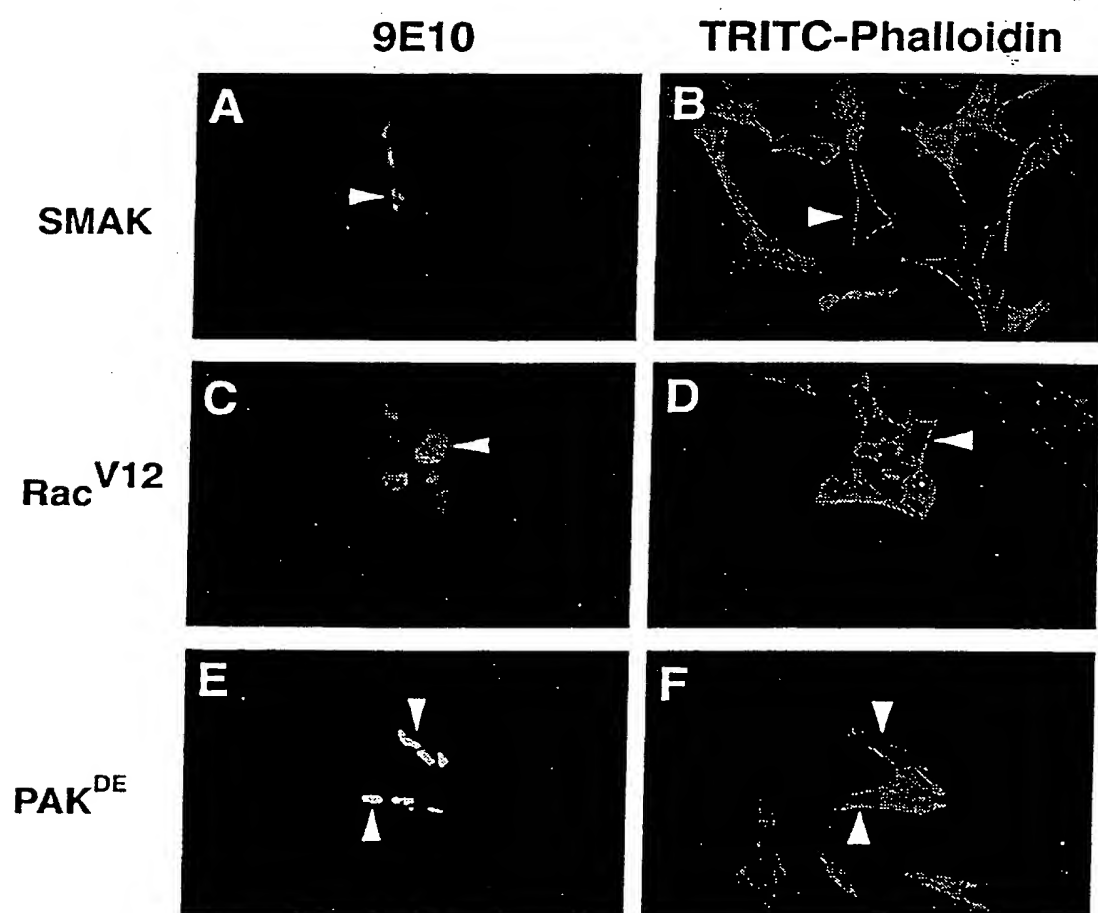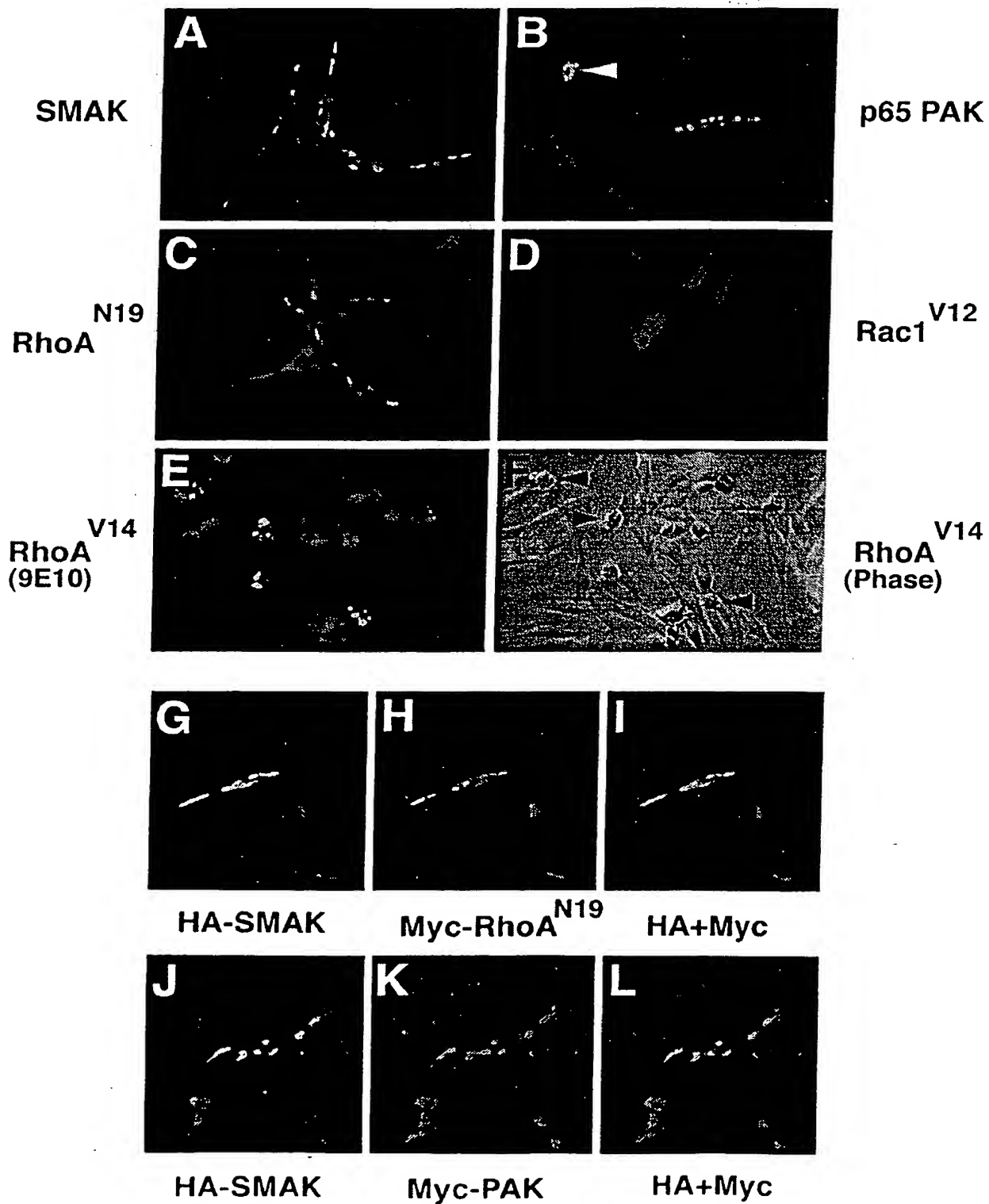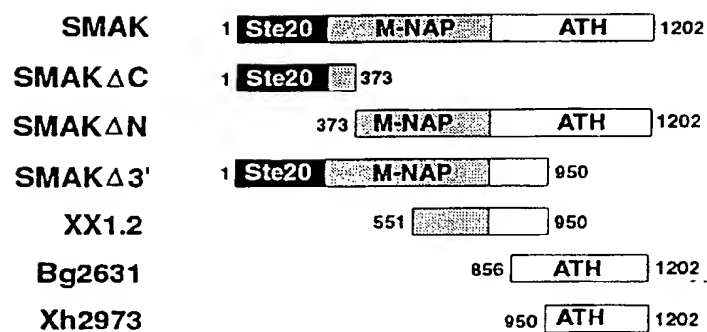
FIGURE 13

FIGURES 14A-J

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FIGURES 15A-E

A

-	-	+	+	+	-	-	-	-	rCaspase 3
-	-	-	-	-	-	+	+	+	Rat1-Myc lysate
-	-	-	-	+	-	-	-	+	Z-DEVD-fmk

WT D436N WT D436N WT WT WT D436N WT

220
133
93
67
56
45

1 2 3 4 5 6 7 8 9

B

Rat1-Myc/ER +
2 μM β-Estradiol

0   8   12   16   24   Hours

220
133
93
67
56
45

1   2   3   4   5

Western anti-SMAK1

C

Rat1-Myc/ER +
2 μM β-Estradiol

NIH3T3

TNF-α+PDC
UV-4h
UV-15h

Hours   0   8   12   16   24   C

56

1   2   3   4   5   6   7   8   9

Western anti-SMAK2

D

WT   D436N   Δ3'   ΔC

-   +   -   +   -   +   -   rCaspase3

◄ H1

1   2   3   4   5   6   7

IP   Kinase Assay

E

293 SMAK D436N Δ3' K63R ΔC

220

93

1   2   3   4   5   6

Western anti-Myc